Plant Physiol. (1992) 99, 1118–1123
0032-0889/92/99/1118/06/$01.00/0

Received for publication August 15, 1991
Accepted February 3, 1992

Effect of Phloem-Translocated Malate on NO_3^- Uptake by Roots of Intact Soybean Plants[1]

Bruno Touraine*, Bertrand Muller, and Claude Grignon

Biochimie et Physiologie Végétales, Institut National de la Recherche Agronomique, Ecole Nationale Supérieure d'Agronomie, Centre National de la Recherche Scientifique URA 573, 34060 Montpellier Cedex 1, France

ABSTRACT

In soybean (*Glycine max* L. Merr. cv Kingsoy), NO_3^- assimilation in leaves resulted in production and transport of malate to roots (B Touraine, N Grignon, C Grignon [1988] Plant Physiol 88: 605–612). This paper examines the significance of this phenomenon for the control of NO_3^- uptake by roots. The net NO_3^- uptake rate by roots of soybean plants was stimulated by the addition of K-malate to the external solution. It was decreased when phloem translocation was interrupted by hypocotyl girdling, and partially restored by malate addition to the medium, whereas glucose was ineffective. Introduction of K-malate into the transpiration stream using a split root system resulted in an enrichment of the phloem sap translocated back to the roots. This treatment resulted in an increase in both NO_3^- uptake and C excretion rates by roots. These results suggest that NO_3^- uptake by roots is dependent on the availability of shoot-borne, phloem-translocated malate. Shoot-to-root transport of malate stimulated NO_3^- uptake, and excretion of HCO_3^- ions was probably released by malate decarboxylation. NO_3^- uptake rate increased when the supply of NO_3^- to the shoot was increased, and decreased when the activity of nitrate reductase in the shoot was inhibited by WO_4^{2-}. We conclude that *in situ*, NO_3^- reduction rate in the shoot may control NO_3^- uptake rate in the roots via the translocation rate of malate in the phloem.

The close relationship between NO_3^- uptake by roots and alkalinization of the rhizosphere is well documented (5, 15, 17, 18, 21). This alkalinization maintains the balance of absorbed charges, whereas the NO_3^--fed plants take up more inorganic anions than cations. It may represent either a H^+ influx into the root cells or a true OH^- efflux, or a HCO_3^- efflux. In the plant cells, OH^- ions are released in the cytoplasm when NO_3^- ions are reduced (19). When NO_3^- reduction occurs in the shoot, the alkalinization of the cytoplasm is thought to be prevented by the biochemical pH-stat, which synthesizes malate ions in response to the pH increase (7, 12). It has been proposed that carboxylate ions may be translocated through the phloem to the root. Thereafter, their decarboxylation would release HCO_3^- ions, which can be extruded into the external solution, balancing the excess anion uptake occurring with NO_3^--fed plants (4, 8). The cycling of K^+ ions between shoot and roots balances the transport of carboxylate ions in the phloem and NO_3^- ions in the xylem (4). A great amount of research has addressed the validity of this model, and more generally the fate of the anion charge released during NO_3^- reduction (*e.g.* 1, 13, 14, 16, 23, 24). In a previous paper (21), we showed that the cycling scheme occurs in NO_3^--fed soybean (*Glycine max* L. Merr. cv Kingsoy) during the vegetative growth phase. The shoot was the site of more than 90% of total NO_3^- reduction, and most of the OH^- equivalents that should have been produced during NO_3^- assimilation were excreted. Moreover, the excretion rate of OH^- equivalents predicted by this calculation was equal to the monitored alkalinization rate of the nutrient solution, and it balanced well the difference between the uptake rates of anions and cations (21). Furthermore, the OH^- equivalents excreted by the roots originated from the carboxylates produced in the shoot, which implies that the negative charge released by NO_3^- reduction in the shoot was transported to the roots through the phloem.

It has been proposed that the model described above plays a regulatory role: the translocation rate of carboxylates in the phloem would be a means by which NO_3^- reduction rate in the shoot controls NO_3^- uptake rate by the roots (4, 8). The present paper deals with this hypothesis.

MATERIALS AND METHODS

Plant Material

Soybean seeds (*Glycine max* L. Merr. cv Kingsoy) were germinated between wet paper towels at 25°C in the dark. Three days later, seedlings were transferred to aerated nutrient solution (10 L for 20 plants) and the plants cultivated in a growth chamber. Temperature was regulated at 25: 20°C during the 14:10 h light:dark cycle. Fluorescent lamps provided a PPFD of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height. The RH was maintained between 70 and 75%.

The culture solution consisted of 2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 0.1 mM Fe-NH_4-EDTA, 50 μM KCl, 30 μM H_3BO_3, 5 μM $MnSO_4$, 1 μM $ZnSO_4$, 1 μM $CuSO_4$, and 0.1 μM $(NH_4)_6Mo_7O_{24}$. The pH of fresh solutions was 5.5. Solutions were renewed every 3 or 4 d in order to minimize pH shift (less than 0.5 pH unit) and nutrient depletion.

Experiments were performed 15 to 18 d after transferring the plants to the growth chamber (18–21 d after sowing). At this stage, the two unifoliolate and the first trifoliolate leaves were mature, the second trifoliolate leaf was fully expanded, and the third trifoliolate leaf had just emerged.

[1] B.M. was supported by Ministère de l'Agriculture, Direction Générale de l'Enseignement et de la Recherche (grant 90121).

Measurement of the Net NO_3^- Uptake Rate

The plants were installed in the experimental conditions the day before the experiment and were left in continuous light until the end of the experiment. The nutrient medium used over the experimental period contained 0.5 mM KNO_3 and 0.1 mM $CaSO_4$, and was aerated. The pH was maintained at 5.5, either by addition of 5 mM Mes-Tris buffer or by a pH-stat system, which consisted of a pH meter (605 pH meter from Metrohm, Switzerland), a control unit (614 Impulsomat from Metrohm), and an automatic burette (655 Dosimat from Metrohm). Using this equipment, the pH was maintained by delivery of 10 mN H_2SO_4 by volumes in the microliter range as soon as the pH shifted of 0.02 pH unit.

The net NO_3^- uptake rate was calculated from the NO_3^- depletion in the solution and corrected for volume variation. For the experiments presented here, the decrease in volume of the solution accounted for 20% or less of the total calculated NO_3^- depletion. Hence, the fluctuations in transpiration rate could not have had a great effect on the data obtained in this study.

In some experiments, phloem translocation was interrupted by steam girdling of the hypocotyl 1 cm above the root junction. Care was taken to ensure that the length of the scalded region did not exceed 0.5 cm and to prevent stem bending. The efficiency of this treatment to block phloem translocation to the roots has been checked with 6(5)carboxyfluorescein (11).

Split Root Experiments

Three plants were used with about two-thirds of their root systems (receiver roots) in 1 L of 0.5 mM KNO_3 plus 0.1 mM $CaSO_4$, and the other third (donor roots) in 600 mL of 0.1 mM $CaSO_4$. At the start of the experiment, the donor roots were cut 5 to 10 cm from their tips. Two or 3 h later, as the treatment was applied to the donor roots, they were recut 2 to 3 cm above the section. Control plants were manipulated in the same manner. The experiments were performed simultaneously with two or three batches of plants treated independently and two batches of control plants. All measurements were in triplicate.

In one split root experiment, the receiver roots of one plant were enclosed in an airtight pot containing 250 mL of the experimental solution aerated with an atmosphere cycling in a closed circuit. The volume of the circuit was sufficient to avoid hypoxy, and the CO_2 content was maintained at a very low level by a CO_2 trap. To estimate the excretion rate of volatile C (CO_2 + HCO_3^-) from the receiver roots, the trap was periodically isolated for 10 min, and the increase of the CO_2 concentration in the atmosphere of the circuit was measured with an IRGA (Li-Cor 6200).

Phloem Exudates

The petioles of the first and second trifoliolate leaves were cut with a razor blade in 20 mM Na-EDTA, pH 7. The petioles were washed in an identical solution, and the leaves were incubated 4 h in the dark and saturated humidity, with the excised sections of the petioles immersed in 5 mM Na-EDTA pH 7 (1.5 mL for two leaves).

EDTA was removed from the exudates by precipitation. For this purpose, after addition of 30 μL of 1 N HCl, the solution was incubated at −20°C for 1 h, thawed at 5°C overnight, and centrifuged at 5000*g* for 10 min. The exudates were purified thereafter for the assay of carboxylic acids as described by Touraine and Astruc (20).

Analytical Methods

NO_3^- was colorimetrically assayed after diazotation of the NO_2^- obtained by reduction in a cadmium column using an automatic analyzer. Carboxylic acids were determined using a dual-column HPLC separation (20).

The NO_3^- uptake data are expressed as micromoles of NO_3^- absorbed per hour and per root dry weight (receiver roots exclusively for split root experiments).

RESULTS

Effect of Malate in the Medium

A concentrated solution of K-malate (pH 5.5, 1.66 K^+:malate molar ratio) was added to the medium to obtain a final concentration of 1 mM malate. Within 1 h, the net NO_3^- uptake rate was increased to a high level that remained steady for at least several hours (Fig. 1). The stimulation of NO_3^- uptake was usually more than 100%, and was also observed in the presence of 50 $mg \cdot L^{-1}$ chloramphenicol (Fig. 1).

Heat girdling of the stem below the unifoliolate leaves led to a quasi-complete inhibition of NO_3^- uptake in 3 to 5 h (Fig. 1). The NO_3^- uptake did not resume during the following hours. Addition of 1 mM malate to the medium restored,

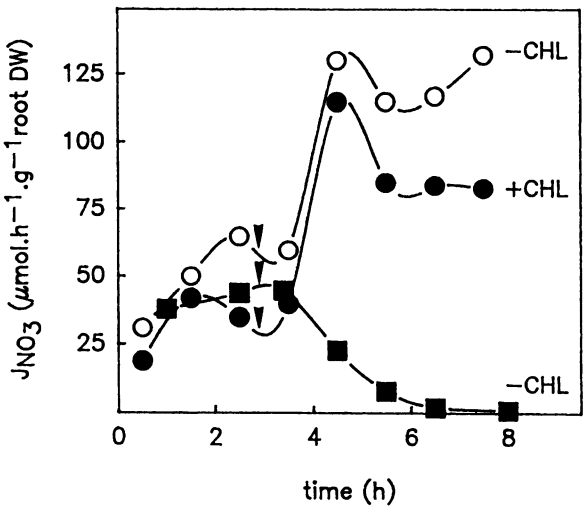

Figure 1. Effects of K-malate addition to the root medium and of hypocotyl girdling on the net NO_3^- uptake rate of soybean plants. The plants were transferred to 0.5 mM KNO_3 plus 0.1 mM $CaSO_4$ 3 d before the experiment, and have been maintained in continuous light for 1 d. Two plots (●, ○) represent the effect of the addition of 1 mM K-malate (arrows) on NO_3^- uptake of intact plants. One plot (■) represents the effect of phloem transport interruption by hypocotyl girdling (arrow) in the absence of malate in the medium. Fifty milligrams per liter chloramphenicol was added (+Chl) or not (−Chl).

at least partially, the net NO_3^- uptake rate, whereas 100 mM glucose did not (data not shown).

Split Root Experiments

These experiments were intended to supply the leaves with NO_3^- or malate (or another compound) while bypassing the part of the root system under examination. The objective was to increase the amount of malate in the leaves, which would result in an increased rate of malate phloem loading and delivery to the roots. To raise the amount of NO_3^- or malate supplied to the receiver roots in this way, we cut the donor roots at about 10 cm from the tip. This operation should have provided also a direct access to the xylem vessels, minimizing malate or NO_3^- metabolism in the donor roots. Table I shows that addition of K-malate to the medium of the donor roots resulted in a significant increase in malate content of phloem exudates. The treatment did not modify the amount of other carboxylates recovered in phloem exudates. These results demonstrate that the approach used allows the supply of solutes to the shoot and then to the receiver roots through the phloem pathway.

Effect of the Supply of Malate to the Shoot

Providing the donor roots with 10 mM K-malate caused an increase in NO_3^- uptake by the receiver roots after a delay of about 2 h (Fig. 2). By contrast, 12.5 mM K_2SO_4 or 25 mM KH_2PO_4 supply to donor roots did not affect NO_3^- uptake by the receiver roots (data not shown).

We had simultaneously measured the net NO_3^- uptake rate and the net excretion rate of volatile C (CO_2 + HCO_3^-) in experiments in which the receiver roots of a plant were enclosed in an airtight pot. Supply of K-malate to the donor roots resulted in a stimulation of both NO_3^- uptake and C excretion. As an example, in one experiment where a 10 mM K-malate pulse was applied to the donor roots from 1.5 to 6 h after the beginning of measurements, the NO_3^- uptake rate increased from 2.8 $\mu mol \cdot h^{-1} \cdot plant^{-1}$ for the period from 0 to 3 h, to 7.1 $\mu mol \cdot h^{-1} \cdot plant^{-1}$ for the period from 3 to 10 h (Fig. 3). The shape of the plot of net NO_3^- uptake rate *versus* time was identical to those of Figure 2. The volatile C excretion rate from the roots was 13.8 $\mu mol \cdot h^{-1} \cdot plant^{-1}$ between 0 and 3 h, and increased to 19.3 $\mu mol \cdot h^{-1} \cdot plant^{-1}$ after 3 h (Fig. 3). The increments of net NO_3^- uptake rate and C excretion rate were similar (Fig. 3). Titration of the receiver roots solution before and after the experiment shown in Figure 3 showed that the amount of OH^- equivalents excreted by the receiver roots was 25 μmol/plant.

Table I. *Effect of 10 mM K-Malate Supply to the Donor Roots of Soybean Plants on the Organic Acid Composition of Phloem Exudates*

This experiment is similar to those in Figure 2. Malate was supplied 4 h before the exudates were collected. Each exudate was collected in EDTA from the cut petioles of the first trifoliolate leaf.

Organic Acid	Amount in the Exudates	
	Control plants	Malate-treated plants
	nmol/exudate[a]	
Malic	215 ± 25	301 ± 36
Malonic	165 ± 15	171 ± 24
Citric	32 ± 10	29 ± 10
Succinic	78 ± 15	84 ± 22
Fumaric	165 ± 29	180 ± 16
Total	652 ± 27	768 ± 29

[a] Mean ± SD (n = 5).

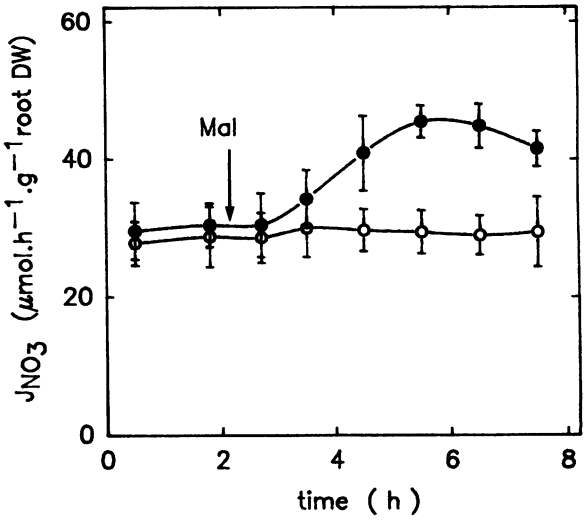

Figure 2. Effect of K-malate introduction into the transpiration flow on the net NO_3^- uptake rate of soybean plants. Each plot represents the pattern of NO_3^- uptake by a part of the root systems (receiver roots) of three plants from 0.5 mM KNO_3 plus 0.1 mM $CaSO_4$. At the start of the experiment, the other roots of the plants were fed with 0.1 mM $CaSO_4$ only. At the time indicated by the arrow, 10 mM K-malate was added to this latter solution (treated plants) or not (control plants). ●, Treated plants; ○, control plants. Data are means ± SD (four experiments).

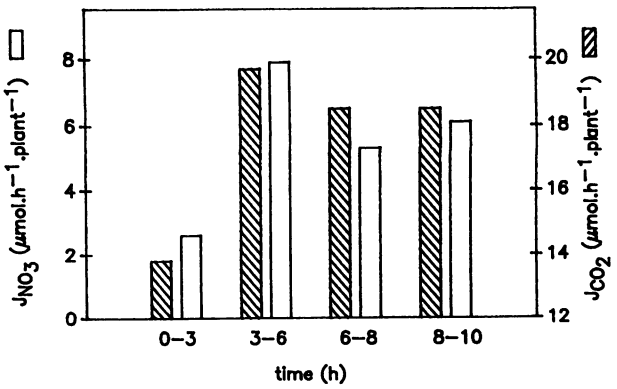

Figure 3. Effect of K-malate introduction into the transpiration flow on the net NO_3^- uptake rate and the C excretion by roots of intact soybean plants. Details are the same as in Figure 2, except that a single plant was used and that the receiver roots were enclosed in an airtight pot. Ten millimolar K-malate was added at 1.5 h.

Effects of the Supply of NO_3^- and WO_4^{2-} to the Shoot

When 25 mM KNO_3 was supplied to the cut donor roots, NO_3^- uptake by the receiver roots was stimulated after a 1 to 2 h delay (Fig. 4). Tungstate, an inhibitor of nitrate reductase activity, supplied to the donor roots as 7.5 mM Na_2WO_4, led to a progressive decrease of the net NO_3^- uptake rate (Fig. 5). The 50% inhibition was reached within 7 to 8 h. Feeding the donor roots with tungstate for 16 h resulted in a 80 ± 7% inhibition of the net NO_3^- uptake rate by receiver roots (mean ± SD of six replicates, data not shown).

DISCUSSION

NO_3^- Uptake Is Dependent on Availability of Malate to Roots

Addition of K-malate to the uptake medium caused a marked increase in the rate of NO_3^- uptake (Fig. 1). The presence of malate in a solution might have favored the proliferation of microorganisms, which might participate to the consumption of NO_3^-. However, when the plants were withdrawn from the pots, the decrease in NO_3^- concentration of the medium ceased. Moreover, the shape of the plot and the level of NO_3^- uptake stimulation caused by the addition of malate were not modified by the presence of chloramphenicol (Fig. 1), an antibiotic that succeeded in limiting the development of microorganisms in the solution for 10 h at least (6). We therefore conclude that the supply of K-malate to the roots was effective in stimulating the net NO_3^- uptake rate.

Malate Provided to Roots by the Phloem Stimulates NO_3^- Uptake

An almost complete inhibition of NO_3^- uptake was observed upon the interruption of phloem translocation to the roots when the hypocotyl was girdled (Fig. 1). The girdling should have caused an energy shortage in the roots by interrupting sucrose translocation, and therefore might have inhibited ion transport systems nonspecifically. This may be expected from studies of the effects of interrupting shoot-to-root translocation on transmembrane potential difference of root cells and on net H^+ exchange rates between the roots and the external solution. Ringing the stem of intact sunflower plants or severing the roots from the shoot resulted in a depolarization of the plasma membrane of root cortical cells as effective as metabolic inhibitor application (10). The transmembrane potential difference was restored by adding 25 mM glucose to the medium (10). In a previous paper, we showed that the net H^+ excretion by roots of intact soybean plants fed with NO_3^--deprived medium was inhibited by heat girdling of the hypocotyl and restored by addition of 100 mM glucose to the medium (21). This indicates that the H^+ pump activity depends on a continuous supply of shoot-borne carbohydrates to the roots, and that it can be restored in roots of girdled plants by glucose addition to the external solution. By contrast, the net OH^- excretion monitored in a NO_3^--containing medium was not restored by 100 mM glucose addition after previous interruption by hypocotyl girdling (21). This is in agreement with the prediction that the negative charges excreted in the medium originated mainly from the shoot, where they were generated by NO_3^- reduction (21). As for the excretion of OH^- equivalents, 100 mM glucose addition to the medium did not succeed in restoring the net NO_3^- uptake rate after inhibition by girdling. By contrast, the addition of 1 mM malate to the medium did restore both the net NO_3^- uptake rate and the net excretion rate of OH^- equivalents. This finding suggests that NO_3^- uptake was under the control of malate imported from the leaves, and that it was coupled to OH^- equivalents excretion.

Feeding the donor roots with 10 mM malate should have resulted in a large increase in carboxylate supply to the leaves. As expected, this treatment increased the malate content of phloem exudates of petioles (Table I). Since the excised petioles were thoroughly rinsed before collection and the exudation rate was steady for several hours (data not shown), we can assume that the collected exudates were not contaminated with xylem sap. We concluded that the supply of K-malate to the donor roots resulted in a higher transport rate

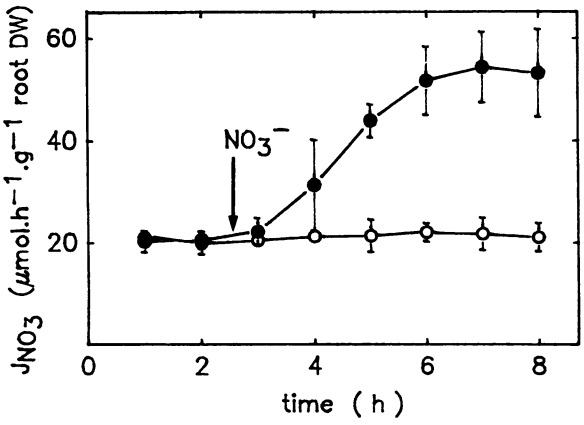

Figure 4. Effect of KNO_3 introduction into the transpiration flow on the net NO_3^- uptake rate of intact soybean plants. Details are the same as in Figure 2, except that 25 mM KNO_3 was supplied at the time indicated by the arrow instead of K-malate. ●, Treated plants; O, control plants. Data are means ± SD (three experiments).

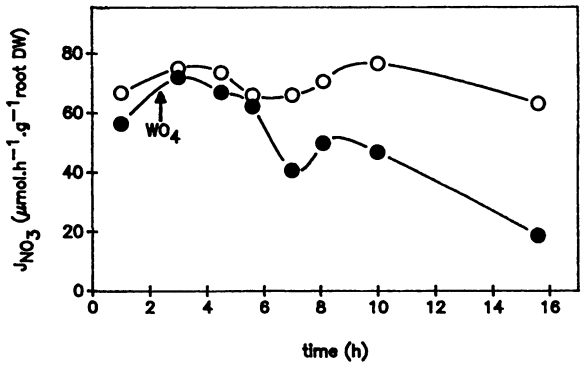

Figure 5. Effect of Na_2WO_4 introduction into the transpiration flow on the net NO_3^- uptake rate of intact soybean plants. Details as in Figure 2, except that 7.5 mM Na_2WO_4 was supplied at 2.5 h (arrow) instead of K-malate. ●, Treated plants; O, control plants.

of this compound to the receiver roots. Thus, the increase in the net NO_3^- uptake rate by the receiver roots (Fig. 2) must have corresponded to a stimulating effect of phloem-translocated malate. Indeed, the stimulation of NO_3^- uptake by K-malate was certainly not due to the effect of K^+ ions, because we observed that addition of K_2SO_4 or KH_2PO_4 at higher concentrations to the donor roots solution had no effect on NO_3^- uptake.

Supplying the shoot with malate through the transpiration stream stimulated the volatile C excretion rate at the same time as the NO_3^- uptake rate. The increments of these two fluxes were simultaneous and of the same magnitude (5.5 and 4.3 $\mu mol \cdot h^{-1} \cdot plant^{-1}$, respectively, in the experiment shown in Fig. 3). These results support the hypothesis that the decarboxylation of shoot-borne malate in the roots produces HCO_3^- ions, which are exchanged for NO_3^- ions taken up (4). In the uptake solution, the HCO_3^- ions would release both volatile C and OH^- equivalents. But, after feeding the leaves with malate via the donor roots, the increment of the alkalinization rate of the medium was always markedly lower than the increments of both the volatile C excretion rate and the NO_3^- uptake rate (22). For instance, in the experiment shown in Figure 3, the amount of NO_3^- that would have been taken up in the absence of malate supply to the donor roots may be estimated at 28 $\mu mol \cdot plant^{-1}$ (28 $\mu mol \cdot h^{-1} \cdot plant^{-1}$ during the 0- to 3-h period multiplied by the 10-h duration of the experiment). If we assume that the ratio of OH^- equivalents excretion rate to NO_3^- uptake rate is 1:2 (21), the amount of OH^- equivalents released in the medium for the uptake of these 28-μmol NO_3^- will be 14 μmol. Because 25-μmol OH^- equivalents were excreted during the whole experiment, only $25 - 14 = 11$ μmol were associated with the malate treatment. This figure is only one-third of the amount of NO_3^- taken up and one-fourth of the amount of volatile C excreted as consequence of the malate treatment ($4.3 \times 7 = 30.1$ and $5.5 \times 7 = 38.5$ μmol, respectively). To explain these differences, one may imagine that part of HCO_3^- excretion coupled to NO_3^- uptake was balanced by H^+ excretion, which would have led to an increase in K^+ uptake.

To summarize, despite the difference between OH^- and C net excretion rates, our results are in favor of a stimulation of NO_3^- uptake coupled to the excretion of HCO_3^- originating from decarboxylation of shoot-borne malate. This conclusion is in agreement with the concomitant increase in malate accumulation and decrease in C excretion observed in roots of tobacco when NO_3^- was withdrawn from the external solution (4).

Modifications of the NO_3^- Reduction Rate in Shoot Affect NO_3^- Uptake

In soybean, the NO_3^- supply through the transpiration stream is a limiting factor for NO_3^- reduction in leaves, even if they have previously accumulated large amounts of NO_3^- (9). Thus, in our experiments, the main part of the NO_3^- supplied to the leaves via the donor roots would have been reduced, and the carboxylate synthesis in the shoot would have been increased. The observed stimulation of the net NO_3^- uptake rate (Fig. 4) suggests that the carboxylates were then transported to the roots and caused the same effect as the malate artificially supplied to the shoot in the previous experiments (Fig. 2 and Table I).

WO_4^{2-} inhibited nitrate reduction, presumably owing to the synthesis of inactive nitrate reductase after incorporation of W into the enzyme in place of Mo (26). When applied to excised organs of corn, WO_4^{2-} caused a progressive decay of nitrate reductase activity, with $k_{0.5}$ values of 6 to 8 h (3). In soybean, WO_4^{2-} led to a complete loss of the NO_3^--induced nitrate reductase activity in leaves when added to the nutrient solution for the whole duration of the culture (2). Thus, the inhibition of the net NO_3^- uptake rate in receiver roots caused by supplying the donor roots with Na_2WO_4 (Fig. 5) is in agreement with the hypothesis that NO_3^- reduction rate in the shoot affects NO_3^- uptake rate by the roots. Furthermore, a direct effect of WO_4^{2-} in the receiver roots was unlikely to occur since there is almost no export of this anion from the leaves (25).

In conclusion, the results presented in this paper indicate that (a) supplying malate to the roots, either by addition to the external solution or by increasing artificially its transport from the shoot in the phloem, resulted in an increase of net NO_3^- uptake rate, and (b) modifications of the NO_3^- reduction rate in the shoot led to similar modifications of the net NO_3^- uptake rate by roots. This sustains the hypothesis that phloem translocation of carboxylate anions produced during NO_3^- reduction in the shoot controls NO_3^- uptake by the roots. This integration of NO_3^- acquisition in the roots and NO_3^- consumption in the shoots might constitute the basis of a regulatory process favoring the maintenance of a NO_3^- reserve in the leaves in addition to NO_3^- for assimilation.

ACKNOWLEDGMENTS

We thank Suzette Astruc for her technical assistance and Dr. Nicole Grignon for 6(5)carboxyfluorescein experiments.

LITERATURE CITED

1. **Allen S, Raven JA** (1987) Intracellular pH regulation in *Ricinus communis* grown with ammonium or nitrate as N source: the role of long distance transport. J Exp Bot **38:** 580–596
2. **Aslam M** (1982) Differential effect of tungsten on the development of endogenous and nitrate-induced nitrate reductase activities in soybean leaves. Plant Physiol **70:** 35–38
3. **Aslam M, Oaks A** (1976) Comparative studies on the induction and inactivation of nitrate reductase in corn roots and leaves. Plant Physiol **57:** 572–576
4. **Ben Zioni A, Vaadia Y, Lips SH** (1971) Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiol Plant **24:** 288–290
5. **Breteler H** (1973) A comparison between ammonium and nitrate nutrition of young sugar-beet plants grown in nutrient solutions at constant acidity. 1. Production of dry matter, ionic balance and chemical composition. Neth J Agric Sci **21:** 227–244
6. **Davidian JC, Soler A, Grignon C** (1984) Development of H^+ extrusion by barley roots after their excision. Physiol Veg **22:** 163–170
7. **Davies DD** (1986) The fine control of cytosolic pH. Physiol Plant **67:** 702–706
8. **Dijkshoorn W, Larthwell DJ, De Wit CT** (1968) Temporal changes in carboxylate content of rye grass with stepwise change in nutrition. Plant Soil **29:** 369–390
9. **Gojon A, Wakrim R, Passama L, Robin P** (1991) Regulation of

NO_3^- assimilation by anion availability in excised soybean leaves. Plant Physiol **96:** 398–405

10. **Graham RB, Bowling DJF** (1977) Effect of the shoot on the transmembrane potentials of root cortical cells of sunflower. J Exp Bot **28:** 886–893
11. **Grignon N, Touraine B, Durand M** (1989) 6(5)carboxyfluorescein as a tracer of phloem sap translocation. Am J Bot **76:** 871–877
12. **Hiatt AJ** (1967) Relationship of cell pH to organic acid change during ion uptake. Plant Physiol **42:** 294–298
13. **Israël DW, Jackson WA** (1982) Ion balance, uptake, and transport processes in N_2-fixing and nitrate- and urea-dependent soybean plants. Plant Physiol **69:** 171–178
14. **Keltjens WG** (1981) Absorption and transport of nutrient cations and anions in maize roots. Plant Soil **63:** 39–46
15. **Keltjens WG, Nijenstein JH** (1987) Diurnal variations in uptake, transport and assimilation of NO_3^- and efflux of OH^- in maize plants. J Plant Nutr **10:** 887–900
16. **Kirkby EA, Armstrong MJ** (1980) Nitrate uptake by roots as regulated by nitrate assimilation in the shoot of castor oil plants. Plant Physiol **65:** 286–290
17. **Macduff JH, Hopper MJ, Wild A, Trim FE** (1987) Comparison of the effects of root temperature on nitrate and ammonium nutrition of oilseed rape (*Brassica napus* L.) in flowing solution culture. II. Cation-anion balance. J Exp Bot **38:** 1589–1602
18. **Marschner H, Römheld V** (1983) *In vivo* measurement of root-induced pH changes at the soil-root interface: effect of plant species and nitrogen source. Z Pflanzenphysiol **111:** 241–251
19. **Raven JA** (1985) pH regulation in plants. Sci Prog **69:** 495–509
20. **Touraine B, Astruc S** (1990) Purification and dual-column HPLC determination of carboxylic acids in tissues, phloem and xylem saps of soybean plants. Chromatographia **30:** 388–392
21. **Touraine B, Grignon N, Grignon C** (1988) Charge balance in NO_3^--fed soybean. Estimation of K^+ and carboxylate recirculation. Plant Physiol **88:** 605–612
22. **Touraine B, Grignon N, Grignon C** (1990) Interaction between nitrate assimilation in shoots and nitrate uptake by roots of soybean (*Glycine max*) plants: role of carboxylate. Plant Soil **124:** 169–174
23. **Van Beusichem ML, Baas R, Kirkby EA, Nelemans JA** (1985) Intracellular pH regulation during NO_3^- assimilation in shoot and roots of *Ricinus communis*. Plant Physiol **78:** 768–773
24. **Van Beusichem ML, Kirkby EA, Baas R** (1988) Influence of nitrate and ammonium nutrition on the uptake, assimilation, and distribution of nutrients in *Ricinus communis*. Plant Physiol **86:** 914–921
25. **Wolterbeek HT, De Bruin M** (1986) The import and redistribution of several cations and anions in tomato leaves. J Exp Bot **37:** 331–340
26. **Wray JL, Filner P** (1970) Structural and functional relationships of enzyme activities induced by nitrate in barley. Biochem J **119:** 715–725